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FROM THE JOURNAL OF AGRICULTURAL SCIENCE,
VOLUME XIX. PART I. 18 JANUARY 1929

CAMBRIDGE
AT THE UNIVERSITY PRESS

PRINTED IN GREAT BRITAIN

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THE ACTION OF CERTAIN CHEMICAL SUBSTANCES ON THE ZOOSPORES OF *PSEUDOPERONOSPORA HUMULI* (MIY. ET TAKAH.) WILS.

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During the summer of 1928 a series of experiments was carried out with the object of testing the suitability of various substances for use on Hops to combat attacks of the Downy Mildew (*Pseudoperonospora Humuli*).

The first experiments were begun early in June. Young hop plants growing in pots were chosen and pairs of leaves at the same node selected. One leaf of each pair was sprayed, using an atomiser, and, when dry, was inoculated with drops of water containing zoospores of the fungus; the other leaf at the node was used as a control and the same inoculum placed on it. In order to provide an atmosphere in which the inoculation drops would not immediately dry up, the plants were placed in a damp chamber[1] erected in a well-lighted and unheated glasshouse. It was considered that the ultimate requirement of a protective spray was that it should prevent the entry of zoospore germ-tubes into the leaf or hop-cone to which the zoospores would be carried by rain.

The first substance to be tested on the growing plant was a proprietary brand of colloidal sulphur, "Ialine[2]," to which 0·5 per cent. saponin was added to secure an unbroken covering of the spray deposit. At the same time, to allow of the effect of the spray material on the zoospores being observed under the microscope, watch glasses were lightly sprayed with the same liquid. After the leaves and the watch-glasses had dried for 24 hours the former were inoculated and the following tests were made with the watch glasses, using the same inoculum.

A drop of water containing the zoospores was placed on the dry spray deposit in one watch glass and a similar drop on the surface of a clean watch glass. It was found that the zoospores, at first actively moving, were unable to exist for more than a minute in the drop of water in the glass sprayed with colloidal sulphur and saponin, whereas in the clean glass the movement was unchecked.

This somewhat surprising result led to a trial of the separate constituents of the fungicide and it was found that neither colloidal sulphur

[1] This measured 12 ft. × 6 ft. × 7½ ft. high, and was made of muslin kept wet by constantly dripping water.

[2] The same brand was used in the subsequent experiments with colloidal sulphur.

alone nor the finest ground sulphur alone, when added to drops of water containing zoospores, had any immediate or strongly inhibitory effect on the activity of the zoospores. It was therefore concluded that the saponin, in addition to acting as a spreader, was actually the fungicide, and one trial of this substance at a strength of 0·5 per cent. showed that it instantly killed all zoospores and caused them to disintegrate in a few seconds, presumably by inducing changes in surface tension. Preliminary trials with soft soap showed that zoospores were similarly unable to exist in 1 per cent. and 0·5 per cent. solutions. The effect was immediate stoppage of movement and a rounding off of the zoospores which either went to the bottom or rose to the surface of the drop. There was then a slow or rapid swelling according to the strength of the solution, and finally a sudden disintegration. Thus, by the addition of a trace of saponin or soap solution, a drop of water with thousands of zoospores in active movement could be immediately cleared of all signs of life.

The rapidity and completeness with which the zoospores were destroyed in the watch-glass tests indicated that the plants sprayed with colloidal sulphur and saponin would not become infected. Of the plants, ten in number, which had been sprayed, the majority showed the first symptoms of infection on the second day on the control leaves, in the form of a yellowish coloration and a glossy appearance of the upper epidermis. After eleven days, when the experiment terminated, all ten unsprayed leaves were heavily infected and bore dense masses of conidiophores. Two of the sprayed leaves each showed a minute area of infection with a single tuft of conidiophores measuring 1 mm. in diameter; the remaining eight leaves were quite healthy and had been efficiently protected by the sulphur and saponin mixture.

The results of this experiment with the living plant and with the watch-glass tests indicated that the fungus is extremely vulnerable in the zoospore stage to the action of soap and saponin. Further experiments were then carried out.

Experiments on the Living Plant.

1. On June 23, 40 hop plants were sprayed: 13 on one leaf each with 0·5 per cent. saponin solution; 13 with a mixture containing 0·5 per cent. colloidal sulphur and 0·5 per cent. saponin, and 14 with 0·5 per cent. lime casein. The 40 opposite control leaves were inoculated first, to allow the spray deposit longer time in which to dry. Inoculation of sprayed leaves was not started until $4\frac{1}{2}$ hours after the spraying had been completed and the lower epidermis appeared quite dry. The inoculum was placed on the leaf by means of a small glass tube, and in doing so care was taken

not to bring the tip of the tube in contact with the leaf[1]. It was found when inoculating the saponin-sprayed leaves that the inoculum spread and ran off the leaf immediately; this necessitated inverting every sprayed leaf and securing it by means of a cleft and bent bamboo standing in the earth of the pot. Lime casein did not prove to be such an effective spreader and the liquid stood on the upturned leaf in large drops[2].

The first infection of the control leaves was seen at the end of the third day and the majority of those sprayed with lime casein showed similar symptoms, viz. large golden brown areas visible on both sides of the leaf.

The plants sprayed with colloidal sulphur and saponin were examined on June 27. Eleven of the sprayed leaves were healthy and two showed small discoloured areas in which mycelium was found. Of the corresponding control leaves only one was healthy, three bore conidiophores and in nine mycelium was found. The tedious process of sectioning the leaves to determine the presence of mycelium was subsequently avoided by placing all leaves, when picked, in damp Petri dishes. When so left overnight infected leaves developed dense grey masses of conidiophores[3]. This method was applied in the case of the 14 leaves sprayed with lime casein, and it was found on June 28 that all had become infected by the fungus. Of the unsprayed (control) leaves, however, only ten were infected. In the case of the 13 leaves sprayed with saponin, all remained healthy whereas the 13 unsprayed all became infected. These results are summarised below:

Exp. 1.

	Colloidal sulphur 0·5 % and saponin 0·5 %	No spray	Lime casein 0·5 %	No spray	Saponin 0·5 %	No spray
Healthy leaves	11	1	0	4	13	0
Infected leaves	2	12	14	10	0	13

2. On June 27 thirty plants were divided into three sets of ten. One, or two, leaves of each plant in one set were sprayed with a mixture

[1] The zoospore suspensions were examined and found active at intervals during the 5 hours required to complete the inoculation and arrangement of the 80 leaves and again after inoculation was finished.

[2] In a test such as this the physical property of saponin is directly connected with the fungicidal effect. The few drops of inoculum spread over the leaf would take into solution more of the active substance than would similar drops when placed on a leaf sprayed with lime casein.

[3] Leaves suspected of containing mycelium were those marked by conspicuous discoloured areas. Presumably the atmosphere in the damp chamber in the glasshouse was sufficiently moist to enable the fungus to infect but not always to fruit on the leaves. In all succeeding glasshouse tests no leaf was recorded as healthy at the end of the experiment until it had been 24 hours in a damp dish and had failed to produce conidiophores.

consisting of 0·5 per cent. soft soap and 0·25 per cent. gelatine; in the next set, with 0·5 per cent. soft soap only, and in the third set with 0·5 per cent. soft soap and 0·25 per cent. flour[1]. The opposite leaf at the node was left unsprayed to provide the control. The inoculations were carried out on the same day, using the methods described above under Exp. 1.

The first signs of discoloration were visible on the upper surface of many control leaves on June 29. On July 4, of the 13 leaves sprayed with soft soap and gelatine, only three were healthy, the remaining ten showing a slight infection by the fungus. Only one (unsprayed) control leaf remained healthy; the other twelve were heavily infected. Eleven of the twelve leaves, sprayed with soap only, became infected, and one remained healthy; the result was the same in the case of the controls, though here the leaves were much more heavily infected, often over the whole lamina. Of the thirteen leaves sprayed with soap and flour-paste, six remained healthy and the other seven became slightly infected in very minute scattered spots; three of the control leaves were healthy and ten were thoroughly infected. The results are summarised below:

Exp. 2.

	Soap 0·5 % and gelatine 0·25 %	No spray	Soap 0·5 %	No spray	Soap 0·5 % and flour[1] 0·25 %	No spray
Healthy leaves	3	1	1	1	6	3
Infected leaves	10	12	11	11	7	10

3. On July 5 thirty plants were divided into three sets of ten. A single leaf on each plant of the first set was sprayed with lime sulphur[2] (1 in 60) + soft soap (1 per cent.); of the second set with Bordeaux mixture[3] (0·25 per cent.) + soft soap (1 per cent.), and of the third set with a mixture of glue (1 per cent.) + soft soap (2 per cent.) + sodium polysulphide solution[4] (0·25 per cent.).

The inoculation of these and of the control leaves was carried out on the same evening. It was found that the drops of inoculum had dried by the following day on the control leaves and on those sprayed with the glue mixture, but on the others not until the second day. Discoloration of the lamina, the first sign of infection, was seen on some control leaves on the second day, and by the fourth (July 9) nearly all control (unsprayed)

[1] The flour was first made into paste and this was added to the soap solution.

[2] The sample of lime sulphur used throughout these experiments contained 21·34 per cent. polysulphide sulphur.

[3] The formula used was: copper sulphate 10 gm., hydrated lime 15 gm. and water 400 c.c., *i.e.* 0·25 per cent. Bordeaux mixture.

[4] The stock solution contained 4·2 per cent. polysulphide sulphur.

leaves were showing light patches above, and were black with conidiophores below. The leaves sprayed with the glue mixture were nearly all normal and healthy; several of those to which lime sulphur and Bordeaux mixture respectively had been applied were slightly mottled as though the fungus had penetrated. No conidiophores, however, were visible, and it was therefore concluded that the markings might perhaps be the result of spray injury, especially because other of these sprayed leaves were smaller than the corresponding control at the same node.

On July 9 all leaves were removed and were placed in damp dishes until the next day when it was found that all ten leaves sprayed with the lime sulphur + soap mixture were free from infection; eight of them, however, were either mottled or were smaller than the controls, showing that they had been adversely affected by the spray. Only one of the ten unsprayed leaves remained healthy; the other nine were heavily infected, up-curling, and black with conidiophores below. All of the ten leaves sprayed with the Bordeaux mixture + soap were healthy, but they were mottled with paler areas in which, on sectioning, no fungus could be found. The ten unsprayed leaves were heavily infected. All those sprayed with the glue mixture were free from infection, but six showed slight brown scorching injury at the extreme apex of the lamina. The sprayed leaves were all heavily infected. The results are summarised below:

Exp. 3.

	Lime sulphur (1 in 60) and soft soap 1 %	No spray	Bordeaux mixture 0·25 % and soft soap 1 %	No spray	Sodium polysulphide solution 0·25 % and glue 1 % and soft soap 2 %	No spray
Healthy leaves	10	1	10	0	10	0
Infected leaves	0	9	0	10	0	10

4. On July 9 one leaf on each of ten plants was sprayed with a mixture of lime sulphur (1 in 100) + soft soap (1 per cent.) and one leaf on each of another ten plants with a mixture of lime sulphur (1 in 100) + gelatine (0·5 per cent.). It was found during the process of inoculation that the water stood in drops on the soap and lime sulphur deposit and spread only very slightly on that of the gelatine and lime sulphur. On the following day all drops were still visible and the control leaves were still moist on the lower surface. On the second day (July 11) a few leaves were seen to be moist, but the majority of the drops of inoculum had dried; the first signs of discoloration of the upper epidermis were appearing on the control (unsprayed) leaves.

On July 14 all leaves were removed and were placed in damp dishes

for examination on the following day when all ten leaves sprayed with lime sulphur and soap were free from infection, but all looked paler in colour than other leaves on the same plants. Six of the ten were slightly smaller than the opposite controls, an effect for which the spray must have been responsible. Only one of the unsprayed leaves remained healthy, the remainder being heavily infected. Three of the leaves sprayed with lime sulphur and gelatine showed on the upper surface minute brown areas which were grey with conidiophores below. The remaining seven were healthy, but three showed light-coloured areas on the upper surface. All of the ten unsprayed leaves were infected, black masses of conidiophores extending over most of the lamina. A summary of these results is given below:

Exp. 4.

	Lime sulphur (1 in 100) and soft soap 1 %	No spray	Lime sulphur (1 in 100) and gelatine 0·5 %	No spray
Healthy leaves	10	1	7	0
Infected leaves	0	9	3	10

With respect to the four preceding experiments the substances chosen for use as protective sprays were in all cases spreaders. In each experiment other substances were added which it was hoped would hold the active agent and make it less readily removable; they had been found useful "stickers" by the watch-glass trials described below, and it was essential that they should be tested on tender leaves of the hop plant in order that any possible injury might be observed. In Exp. 1 no injury resulted from the use of colloidal sulphur as a sticker for saponin. Lime casein, a spreader, proved to be inefficient, whereas saponin used alone provided complete protection against the zoospores. As mentioned above (footnote, p. 187), it is possible that efficiency in spreading the inoculation drop may be connected with the fungicidal power of the spreader. As regards Exp. 2, the general failure to prevent infection by soft soap with or without a sticking agent may be considered as due to the fact that the original concentration of the spray (0·5 per cent.) in relation to the comparatively large drops of inoculum was probably insufficient. It must be remembered, however, that both in the preliminary experiment and in Exp. 1 saponin proved completely effective when applied at the same strength (0·5 per cent.). It is possible that during the length of time taken to complete the inoculation a small number of zoospores might have become rounded off preparatory to germination, and it is not known whether in this state they would be affected by the soap or saponin. In Exp. 3 lime sulphur, Bordeaux mixture and sodium polysulphide were all used as holders for the soap, but their

direct action as fungicides must be taken into consideration. Exp. 4 was designed to compare lime sulphur and soap on the one hand with lime sulphur alone on the other, but it was found that lime sulphur (1 in 100) would not spread sufficiently well. Gelatine as an inactive spreader was consequently employed.

In all these experiments the sprayed leaves were held inverted by means of split bamboos or by wires until the drops of inoculum had dried. The control leaves were not so treated because in the absence of a spreader the water remained sufficiently long on the lower surface without dripping off.

Laboratory Experiments.

Trials were made with various substances to discover whether they were capable of killing zoospores (see (1) below). Wet tests were made (see (2) below) with equal parts of diluted solutions and of the zoospore suspension. It was necessary in most cases, owing to the ready solubility of the fungicidal substances, to find some means of holding them and delaying their solution in water. With this object certain other substances were mixed with them and the liquid either tested immediately or sprayed or dropped on to glass and allowed to dry. In the latter case a standard quantity of the zoospore suspension was applied and removed, the process of application and removal being repeated until a weakening of the killing power was observed. In these tests (see (3) below) the spray deposit was not allowed to dry in the intervals between removal and fresh application of zoospore-containing water.

Some of these tests were also applied to the zoospores of *Phytophthora infestans*.

In all cases control drops from the same pipette were under observation and the zoospores invariably remained active.

(1) *Preliminary trials.*

The following substances were roughly tested in the ways indicated: *sodium silicate, lime casein, colloidal sulphur* and *lime water*. A 0·5 per cent. solution of each of the first three named was used. The liquid was sprayed on a watch glass and allowed to dry. Drops of water containing zoospores were placed on the dry deposit and it was found that neither sodium silicate nor lime casein had any immediate effect. The colloidal sulphur caused some zoospores to come to rest, but many continued to move. A wet test with colloidal sulphur, in which a drop (0·1 c.c.) of a 1 per cent. suspension was diluted to 0·5 per cent. by mixing with an equal drop of water containing zoospores, showed that the zoospores moved about amongst the particles for 12 minutes and then stopped;

many remained motionless but were moving their flagella. A similar test with 0·5 per cent. lime casein was carried out. The zoospores rounded off and then burst after a pause of a few seconds, but diffusion of the active agent into the added drop of water was so slow that an accurate estimation of the time required could not be made. In a watch-glass test with the dried-on deposit of limewater it was noticed that the zoospores were attracted by small fragments at the bottom of the drop. They collected round one or two fragments and remained inactive. *Glycerine.* A trace of this substance added to a drop of water containing zoospores caused instant shrivelling of the zoospores. *Iodine.* By placing a glass bottle-stopper moistened with iodine solution one inch or more away from the microscope stage bearing a slide with a small drop of water with active zoospores, the latter were instantly brought to the surface of the drop where they came to rest and rounded off. This effect was apparently dependent on a current of air sufficiently strong to carry the vapour in the right direction. Iodine in solution (iodine 1 gm., potassium iodide 2 gm., water 300 c.c.) was tested by making 1 c.c. up to 100 c.c. by addition of water. 1 c.c. of this weak solution (0·003 per cent.) was diluted to half strength by addition of an equal volume of zoospore suspension. At this concentration of iodine (0·0016 per cent.) all movement ceased within 5 minutes. The stoppage, which was mostly instantaneous, was followed by slow swelling, and after 10 minutes by bursting of the zoospores. *Bromine.* The extreme sensitiveness of the zoospores to bromine (as to iodine) is shown by the fact that the small amount of bromine vapour which escaped from an ordinary glass-stoppered bottle placed about 5 in. away from the microscope stage was able to arrest and cause disintegration of the zoospores in a drop of water on a slide. *Gelatine.* At a concentration of 0·5 per cent. no effect was produced on zoospores. *Flour paste.* One drop of paste (5 per cent. flour) was added to two drops of inoculum with no effect. "*Sunoco.*" This commercial preparation of a self-emulsifying petroleum oil, at a strength of 0·5 per cent., did not inhibit movement completely but reduced the activity of the zoospores very considerably. *Resin soap.* In a 2·5 per cent. solution zoospores were caused to burst, but 4 minutes were required for their total disappearance. *Caustic soda.* A 0·5 per cent. solution brought about immediate bursting. *Bordeaux mixture.* A wet test, in which 0·125 per cent. Bordeaux was used, resulted in instant stoppage of movement and rapid disappearance of zoospores. The cloudiness of the liquid made observation difficult. *Lime sulphur.* A 0·5 per cent. solution caused immediate stopping and rounding off at the centre of the drop. Zoospores collected in large groups. Particles of free sulphur

coming out of combination in the liquid made it impossible to see the subsequent behaviour of the zoospores.

(2) *Further tests.*

Soft soap. Tests were made at eight different concentrations from 0·5 per cent. to 0·005 per cent., and from the results which are summarised below, it appears that solutions stronger than 0·02 per cent. are completely effective. Reduced activity was immediately evident in all cases. In all dilutions except 0·005 per cent., total stoppage of movement was instantaneous, but in this lowest dilution, 15 minutes elapsed before all zoospores had come to rest.

Effect of soft soap solutions on zoospores.

Percentage soft soap in solution	Swelling	Bursting	
		Begins	Ends
0·5	Instant	Instantly	Instantly
0·25	„	„	„
0·125	Gradual	In few seconds	In few seconds
0·0625	„	„	„
0·03125	Slower	„	„
0·0156	Slow	„	In 60 seconds
0·01	„	In 60 seconds	In 3 minutes
0·005	Very slow	In 6 minutes	In 2 hours

Caustic soda. Zoospores in a solution containing 0·05 per cent. NaOH were affected as they were in soap solutions and all had disintegrated within 60 seconds. In a weaker solution, 0·025 per cent. NaOH, movement was stopped within 60 seconds, a general bursting followed and all zoospores had disappeared in 10 minutes. *Lime sulphur.* Zoospores in 0·25 per cent., *i.e.* 1 in 400, lime sulphur were caused to stop in 30 seconds and to round off at the surface of the drop. Swelling was slow and bursting only started after 8 minutes; in a 0·125 per cent., *i.e.* 1 in 800, solution the stoppage of movement took place in 30 seconds and, as in the former case, the majority rounded off in large groups at the surface of the drop. Even after an hour bursting was not complete, many rounded-off zoospores remaining on the surface. A wet test with a mixture of lime sulphur and soap was carried out and in a solution containing lime sulphur 1 in 400 and soft soap 0·25 per cent.[1] swelling of the zoospores and finally bursting was very slow. In a solution of half this strength (lime sulphur 1 in 800 and soap 0·125 per cent.) the zoospores all rounded off in 5 to 10 seconds. Swelling was gradual and bursting started after 5 minutes. All zoospores had completely disappeared in 10 minutes from the start of the test. This time is considerably more than

[1] The original solution, before dilution, was prepared by mixing 2·0 per cent. soap solution and 1 in 50 lime sulphur solution in equal quantities.

was required in a solution of soap only (0·125 per cent.) as described above, and it would seem that addition of lime sulphur reduced its efficiency.

(3) *Adherent mixtures.*

In a liquid containing soft soap (0·25 per cent.) and gelatine (0·125 per cent.) zoospores were caused instantly to disintegrate. The same effect was produced when flour paste (0·125 per cent. flour) was substituted for gelatine. Watch glasses sprayed with a thin film of the above mixtures were allowed to dry and were then filled with water and emptied three times, after which drops of water with zoospores were run on. The flour mixture was inactive. The gelatine mixture, however, caused slowing up but no killing for about 5 minutes. At that time zoospores at the bottom of the glass swelled up and some burst, but after an hour no more than approximately one-third of the original number had disintegrated. Soft soap alone did not prove to adhere sufficiently well to the watch glass. A drop (unmeasured) of a 1 per cent. solution, when allowed to dry overnight, killed the zoospores in a drop of water (0·1 c.c.) which was placed on the deposit, but when, after this had been drained off, a second drop containing active zoospores was placed on the watch glass no strong fungicidal effect was produced.

A drop (unmeasured) of a solution containing equal parts of 1 per cent. soft soap and 2 per cent. glue was allowed to dry on a watch glass. Zoospores in a drop of water (0·1 c.c.) applied to the deposit stopped movement in 3 minutes and all burst at the end of 10 minutes. After removal of this drop a second was added and a more rapid action followed; movement was instantly stopped and a general bursting of the zoospores began after 5 minutes. In a third drop movement ceased after 3 minutes and the majority of the zoospores had disappeared after 6 minutes. In the fourth drop added the zoospores continued movement for 10 minutes and were not affected even after one hour had elapsed.

A single drop of a solution composed of 40 c.c. of 4 per cent. soft soap, 40 c.c. of 2 per cent. glue, 10 c.c. of sodium polysulphide solution (containing 4·2 per cent. polysulphide sulphur) and 90 c.c. water was allowed to dry on a watch glass and the deposit was tested as before. In the first drop there was instant killing; in the second all had burst within 3 minutes; in the third within 6 minutes; but in the fourth there was only a gradual slowing after 4 minutes and most of the zoospores were not motionless until after 7 minutes.

A mixture of casein and soap was next tested by means of a single drop (0·1 c.c.) allowed to dry on the centre of a watch glass (soft soap 20 gm., casein 10 gm., sodium hydrate (10 per cent.) 50 c.c.; the whole

being made up to 100 c.c. by addition of water). In the first drop (0·1 c.c.) of a zoospore suspension placed on the dry deposit the zoospores were immediately killed; this was run off and a second drop added, and total destruction of the zoospores resulted in $2\frac{1}{2}$ minutes; for the third drop $5\frac{1}{2}$ minutes were required for the same effect, and in the fourth the zoospores were still moving after 10 minutes.

0·1 c.c. of a solution of soap (2 per cent.) with ferrous hydrate (5 per cent.) was tested. When the drop (0·1 c.c.) of the zoospore suspension was added the dried-on deposit readily came away from the glass in a coloured stream, but it did not quickly diffuse through the added water. Zoospores instantly burst when they came in contact with the soap stream; the remainder which had rounded off when the drop was first added burst gradually as diffusion took place. The first drop was drained off and another added. In this diffusion was very slow and 7 minutes elapsed before all movement had ceased and bursting of zoospores became general. In the third drop added movement continued for more than 10 minutes and the test was not proceeded with. It appeared that the ferrous-ferric hydrate was a good holder of the soap, making a viscous mixture, but it did not adhere well to the glass.

One drop (0·2 c.c.) of a solution of resin soap (5 per cent.) with no added sticker was allowed to dry on a watch glass. Zoospores in an equal drop of water stopped movement and all burst within 4 minutes. In the second drop movement was stopped after $3\frac{1}{2}$ minutes, but bursting was delayed until 6 minutes had passed; then it took place quickly and all zoospores had disappeared at the 7th minute. In the third drop movement continued for 10 minutes without any signs of slowing down and the test was not continued.

The fungicides used on the living plant (Exp. 3) were tested on watch glasses by allowing one drop (0·05 c.c.) to dry on. Successive drops of zoospore suspension, each 0·05 c.c., were placed on the spray deposit and drained off after some definite effect on the zoospores had been noted. The deposit was not allowed to dry in the interval between removal of one drop and replacement by another. In this way lime sulphur (1 in 60) and soft soap (1 per cent.) was found to bring zoospores to a standstill even in the eleventh drop added. The time required for complete disintegration was somewhat long (14–20 minutes) in all the drops added.

Bordeaux mixture (0·25 per cent.) and soft soap (1 per cent.) was tested to the eighth drop; it arrested movement more rapidly than the lime sulphur, but the time required for complete removal of zoospores was about the same, *i.e.* up to 20 minutes at the seventh drop. The

results of these experiments, using lime sulphur or Bordeaux mixture in combination with soap, are summarised below:

Effect on zoospores.

Spray deposit	Movement stopped	Swelling	Bursting	
			Begins	Ends
Lime sulphur 1 in 60 and soft soap 1 %:				
Added drop 1	Instantly	Very slow	6 min.	—
„ 2	1·0 min.	„	8 min.	—
„ 3	1·5 min.	„	10 min.	—
„ 4	1·5 min.	„	8 min.	14 min.
„ 5	1·5 min.	„	—	14 min.
„ 6	2·0 min.	„	*	*
„ 7	8·0 min.	„	14 min.	—
„ 8	7·0 min.	„	—	20 min.
„ 9	—	„	—	20 min.
„ 10	—	„	—	14 min.
„ 11	5·0 min.	„	—	—
Bordeaux mixture 0·25 % and soft soap 1 %:				
Added drop 1	Immediately	Slow	—	5 min.
„ 2	„	„	—	7 min.
„ 3	„	„	*	*
„ 4	„	„	—	13 min.
„ 5	„	„	—	11 min.
„ 6	1·0 min.	„	—	14 min.
„ 7	1·0 min.	Very slow	—	20 min.
„ 8	1·0 min.	„	—	15 min.

* Drained off and glass left for half-hour interval.

It is known that salts of aluminium have fungicidal properties (7) and field tests have been made by Kelsall (6) with an aluminium-lime mixture. Wet and dry tests, with and without soft soap, were therefore made on watch glasses with a mixture which had the following composition: hydrated lime 0·25 gm., aluminium sulphate 0·25 gm., soft soap 0·5 gm. and water 100 c.c. This 0·25 per cent. aluminium-lime mixture was first tested without the addition of soap. A drop (0·1 c.c.) was placed in the watch glass and to this was added an equal drop (0·1 c.c.) of the zoospore suspension, making the concentration now 0·125 per cent. aluminium sulphate. The test was repeated five times and on every occasion the zoospores were instantly brought to a standstill and rounded off. Bursting was completed in from $2\frac{1}{2}$ to 5 minutes.

In a similar test repeated three times, using 0·125 per cent. aluminium sulphate and soft soap, as shown above, movement was stopped in 5 seconds and bursting was complete in from $2\frac{1}{2}$ to $4\frac{1}{2}$ minutes.

One drop of the complete mixture, *i.e.* with soap, was allowed to dry on a watch glass for 4 hours. To the dry deposit was added an equal drop (0·5 c.c.) of zoospore suspension. All movement was stopped in 5 minutes at the bottom of the drop where slow swelling then took place;

at the surface, however, movement was continued for much longer (15 minutes). The glass was washed out under running tap water and the white deposit still adhered. After draining off all excess water a second drop (0·05 c.c.) containing zoospores was placed on the watch glass. After a few minutes many went to the bottom but others continued to swim. After half an hour very few remained in movement and their progress was restricted and spasmodic. Many were at the bottom of the drop in contact with the precipitate and were in all stages of swelling and bursting. After two hours all zoospores had disappeared.

Several plants in the Wye College hop garden were sprayed, when fully grown, with aluminium-lime mixture, but the incidence of Downy Mildew on the cones of the unsprayed control hops was so slight in 1928 as to make it impossible to judge the protective power of the mixture on the sprayed cones. It was observed that this mixture on the plants was of a light greyish white colour, that it adhered to the foliage in spite of rains and that it caused no scorching.

Laboratory Experiments with Zoospores of *Phytophthora infestans*.

At the end of August the opportunity was taken to test the reaction of zoospores of the potato blight (*Phytophthora infestans*) to soap and saponin solutions as well as to iodine vapour. Potato leaves with patches of blight were collected on August 27 and were kept in a damp atmosphere till the next day to increase the growth of conidia. By means of a camel-hair brush the spores were placed in watch glasses of rain water which had just been collected during a shower. Compared with the rapid germination of conidia of *Pseudoperonospora Humuli* which require about 1¼ hours at 19° C., those of *Phytophthora infestans* were slow in producing zoospores; at the same temperature (19° C.) 4 hours elapsed from the time of placing the first spores in water to the emergence of zoospores[1]. Four different concentrations of soft soap were employed, the final dilution of each being determined as in the foregoing experiments by using a drop of standard size (0·1 c.c.) and adding to it an exactly similar

[1] In addition to temperature, other factors may be concerned. Thus in the case of *Plasmopara viticola* it has been found (5) that germination takes place in 1 to 8 hours after placing the spores in water. When nutritive solutions are used, zoospore formation is more active at the greater dilutions; the age of the conidium and its condition resulting from previous existence in a wet or dry atmosphere are also to be taken into account. Thus ripe conidia from fairly dry surroundings form zoospores in 6 to 8 hours, but if previously kept in moist air, they form zoospores in 1 to 2 hours. Conidia kept for 2 or 3 weeks form zoospores only after 24 to 48 hours' immersion in water.

drop of zoospore suspension from a pipette. The results are summarised below:

Phytophthora infestans.

Effect on zoospores placed in various concentrations of soft soap.

Soft soap %	Movement stopped	Swelling	Bursting	
			Begins	Ends
0·125	Instantly	Instantly	Instantly	10 sec.
0·0625	,,	,,	,,	4 min.
0·03125	,,	,,	3 sec.	7 min.
0·0156	,,	Gradually	2 min.	12 min.

A similar test with two concentrations of saponin was carried out. In 0·0625 per cent. saponin the zoospores rounded off within 60 seconds and slowly swelled; bursting was almost simultaneous and was completed after 7 minutes. In 0·0156 per cent. saponin, movement was stopped in one minute; the zoospores then slowly swelled and bursting was spasmodical. At the end of 8 minutes a few zoospores remained.

Iodine affected these zoospores in the same way as those of the Hop Downy Mildew. The stopper of a bottle which contained iodine solution was held 2 inches away from a watch glass on which was a single drop (0·2 c.c.) of water containing zoospores. The effect was immediate and zoospores rose to the surface of the drop and came to rest. They then very slowly swelled and started to burst after 3 minutes.

Discussion of Results.

It is clear from the above experiments that the Hop Downy Mildew at the stage when free zoospores have been produced is extremely vulnerable. The zoospores can be exterminated by means of various substances, viz. soap, saponin, glycerine, iodine, bromine, and aluminium-lime mixture. In the case of soap and saponin, and perhaps other substances, it seems possible that the disintegration of zoospores is caused by changes in surface tension. It is somewhat surprising that the lethal action of these substances, specially of soap, on zoospores has not apparently been recorded[1].

The fungicidal power of soap has, however, been recognised; solutions containing 0·5–1·0 per cent. have been used in vineyard practice in Germany against *Botrytis cinerea* (13), and solutions of 5–7 per cent. are stated to control certain powdery mildews, viz. *Sphaerotheca pannosa* (2) and

[1] The action of chemicals on free-swimming zoospores has seemingly been studied by few observers; Wüthrich (14) records the effects of different solutions of metallic salts and certain acids on the free zoospores of *Phytophthora infestans* and *Plasmopara viticola*. Millardet and Gayon (9) studied the effect of lime, copper sulphate, and iron sulphate on the zoospores of *P. viticola*; they remark, "... les conidies et les zoospores qu'elles engendrent sont, à l'égard de ces solutions, d'une sensibilité vraiment prodigieuse."

S. mors-uvae (4, 8). Iodine has been employed for the disinfection of seed oats (12) and bromine for other seeds (1, 11 bis). Experiments by Owen (11) have shown that sclerotia of *Colletotrichum tabificum*[1] are killed after 17 hours' immersion in iodine solutions (0·1 per cent. to 0·45 per cent.). The action of iodine as a disinfectant or as a killing agent in laboratory technique has long been known; the use of iodine vapour for this latter purpose was described by Overton (10) in 1890.

With proof now obtained of the extreme susceptibility of zoospores to soap, which has no disadvantages for use on hops, it is to be hoped that some practical method may be devised whereby this or some such zoospore-killing substance will be utilised during the time (about six weeks) when the cones are developing. At this period great anxiety is experienced by the grower, owing to the fact that the cones may be attacked, turned brown and rendered useless in so short a space of time as two or three days. The ready solubility of soap makes it appear at first sight unsuitable on account of the necessity of replacing it after every shower of rain. This difficulty, which is certainly serious, may possibly be overcome (1) by finding some base capable of holding the soap in a wet spray and making it less readily removed, or (2) by employing a soap-containing dust which on account of its easier application could be put on the hops after every rain, at least during the final week before picking.

The preliminary experiments already made show that a mixture of soap with lime sulphur, for example, has a fairly lasting fungicidal power when tested on glass and was successful in protecting the inoculated leaves of growing plants.

As far as we know there are no soap-containing dusts in use as fungicides or insecticides, but their elaboration should not present any serious difficulty. Although it is well known that other dusts in use as substitutes for wet protective sprays do not adhere so well as the wet sprays, there is a possibility that in the case of the closely crowded and overlapping "petals" of the hop cone a dust might prove as effective as a wet spray[2].

Summary.

1. Both *Pseudoperonospora Humuli* and *Phytophthora infestans* are extremely susceptible in the zoospore stage to the action of weak solutions of soap or saponin. The zoospores are caused to disintegrate suddenly,

[1] Now called *Colletotrichum atramentarium.*

[2] In discussing this subject relative to the Vine Downy Mildew, Delacroix and Maublanc (3) state that copper sulphate powders are inferior to the wet sprays in adherent properties except in the one case where the dusts adhere better to the smooth surface of the grapes.

apparently by changes in surface tension, within 60 seconds, in solutions containing over 0·1 per cent. soft soap. Those of *P. Humuli* are more vulnerable than those of *P. infestans*.

2. The fungicidal action of soap and saponin mixed with certain adherent substances was tested on hop plants.

3. The power of adhesion and the fungicidal efficiency of the mixtures were tested by allowing single drops to dry on the surface of watch glasses and by then adding drops of water containing zoospores.

4. Other substances, *e.g.* aluminium-lime mixture, glycerine, iodine, bromine, were also found to kill zoospores rapidly.

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(*Received November 17th*, 1928.)

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